Histopathology and immunohistochemistry of dermatoborrelioses

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SUMMARY

There are three different dermatological manifestations of LB (dermatoborrelioses, DB), erythema rnigrans (EM), borrelial lymphocytoma (BL), and acrodermatitis chronica atrophicans (ACA). The diagnosis of all three of these DB is primarily made on clinical grounds. Analysis of serum antibodies to *B. burgdorferi* is often unreliable and direct diagnostic methods (cultivation and PCR) are available in specialized laboratories only.

Histopathologic examination of biopsy samples from lesional skin of patients with suspected DB is a very helpful adjunct to the diagnosis. The most important finding in EM is a patchy mononuclear superficial (and deep); perivascular (and interstitial) infiltrate that is composed predominantly of lymphocytes and histiocytes with a variable admixture of plasma cells. There are two histopathologic types of BL, with (follicular type) or without (diffuse/nodular type) follicular structures, resembling the germinal centers of lymph nodes. BL can be confused with well-differentiated nodular lymphoma histologically, but BL is always a pseudolymphoma, thus representing a benign lesion with a polyclonal proliferation of B and T cells. ACA develops clinically from an acute inflammatory phase into a chronic atrophic phase, which is reflected by different histopathologic features with a predominance of infiltrative changes in the early stage and cutaneous atrophy in the late stage. A significant finding in ACA is a patchy to band-like mononuclear infiltrate that is pronounced in the superficial dermis but also present in the deep portion of the dermis. The infiltrate is concentrated around blood vessels, which are often dilated. Immunohistochemical investigation reveals in all forms of DB a mixed mononuclear infiltrate, in which CD8+ cells always outnumber CD4+ cells. Macrophages are found significantly more often in EM patients with associated features than in those without. T cells are prevalent in EM, whereas B cells are prevalent in BL.

KEY WORDS

Lyme borreliosis, *Borrelia burgdorferi*, dermatoborrelioses, erythema migrans, borrelial lymphocytoma, acrodermatitis chronica atrophicans, histopathology, light microscopy, immunohistochemistry

Introduction

Lyme borreliosis (LB), a multisystemic infectious disease that is caused by the spirochete *Borrelia burgdorferi* (Bb), has a complex inflammatory pathology (1) that is reflected by distinct histopathologic features at the site(s) of infection. There are three different dermatological manifestations of LB (dermatoborrelioses, DB), erythema migrans (EM), borrelial lymphocytoma (BL), and acrodermatitis chronica atrophicans

Table. Expression of leukocyte differentiation antigens based on a score of 0-3 in lesional skin of patients with various manifestations of dermatoborrelioses (mean values ± standard deviation)

	CD68 (macrophages)	CD3 (T cells)	CD4 (helper T cells)	CD8 (suppressor T cells)	CD20 (B cells)
EM major	2.5 (±0.7)[ab]	2.1 (±0.7)[d]	0.5 (±0.5)	2.5 (±0.5)[f]	1.0 (±0.6)
EM minor	1.5 (±0.8)	2.3 (±0.4)[de]	0.6 (±0.4)	1.8 (±0.4)[f]	1.4 (±0.9)
BL	2.5 (±0.8)	2.3 (±0.7)	0.5 (±0.0)	2.2 (±1.2)[f]	3.0 (±0.0)[g]
ACA	2.2 (±0.6)[c]	1.8 (±0.9)	0.3 (±0.4)	1.6 (±0.9)[f]	2.0 (±0.9)[h]

a, $p<0.05$ vs CD20+ cells; b, $p<0.05$ vs EM minor; c, $p=0.05$ vs EM minor; d, $p<0.05$ vs CD20+ cells; e, $p<0.05$ vs CD68+ cells; f, $p<0.05$ vs CD4+ cells; g, $p<0.05$ vs EM major and minor; h, $p=0.05$ vs EM major

(ACA). The diagnosis of all three of these DB is primarily made on clinical grounds. Analysis of serum antibodies to Bb by ELISA tests is routinely performed, but is supportive only in ACA, where a positive IgG titer is found in all patients, and in BL, where around 80% of patients are seropositive. In EM, however, serologic diagnosis is very unreliable due to frequent false positive or false negative results and lack of standardization. Moreover, in Europe no single set of criteria for the interpretation of immunoblot analyses results in high levels of sensitivity and specificity (2). Direct diagnostic methods include cultivation of Bb from lesional skin of patients in a Barbour-Stoenner-Kelly medium and detection of Bb specific DNA by polymerase chain reaction (PCR). Cultivation of Bb may reach a sensitivity of 80% under optimal conditions, but is laborious and results are not available before 1-4 weeks after biopsy depending on the multiplication of the spirochetes. PCR from skin biopsy samples is an advantageous technique as it amplifies the usually low amount of Bb specific DNA present, but is available in specialized laboratories only and false positive results due to contamination pose a potential problem. Thus, histopathologic examination of biopsy samples from lesional skin of patients with suspected DB is a very helpful and straightforward adjunct to the diagnosis. The clinical diagnosis of DB can be supported and important differential diagnoses can be excluded primarily by the pattern and cellular composition of the inflammatory infiltrate in the skin lesion. For histopathologic examination of DB, a 4-mm punch biopsy must be obtained under sterile conditions following local anesthesia from the leading edge of the rash in EM, from the central part of the nodule or plaque in BL, and from the area with the most prominent signs of inflammation in ACA. The biopsy specimen has to be placed immediately in 4% formaldehyde/phosphate buffer saline and fixed overnight, dehydrated in graded ethanol and embedded in paraffin. Sections of 4 µm are then cut for histopathology (routine staining with hematoxylin and eosin), and of 5 µm for immunohistochemistry.

The present article describes the histopathologic and immunohistochemical features of the various manifestations of DB and reflects our continuing experience from more than 500 patients biopsied at our department during the last eight years.

Histopathology of erythema migrans

The most important finding in EM is a patchy mononuclear infiltrate of mild to moderate or even severe density depending on the clinical degree of inflammation. The infiltrate is located mostly in the superficial but also in the deeper dermis with a perivascular concentration, although there is also a less intense interstitial infiltrate (Figure 1). Periglandular and perineural infiltrates may occur. The blood vessels around which the infiltrates are accentuated may be dilated but are never damaged. The infiltrate is composed predominantly of lymphocytes and histiocytes with a variable admixture of plasma cells. The number of plasma cells is usually greater in older lesions. In (very) early lesions, a small number of neutrophils and eosinophils may also be present (4), which can make the differentiation to unspecific tick or insect bite reactions difficult. The early skin lesion around a tick bite is characterized by an epidermal blister containing erythrocytes and nuclear debris and a superficial and deep predominantly neutrophilic infiltrate, intermingled with lymphocytes, histiocytes, and eosinophils. This infiltrate is most intense

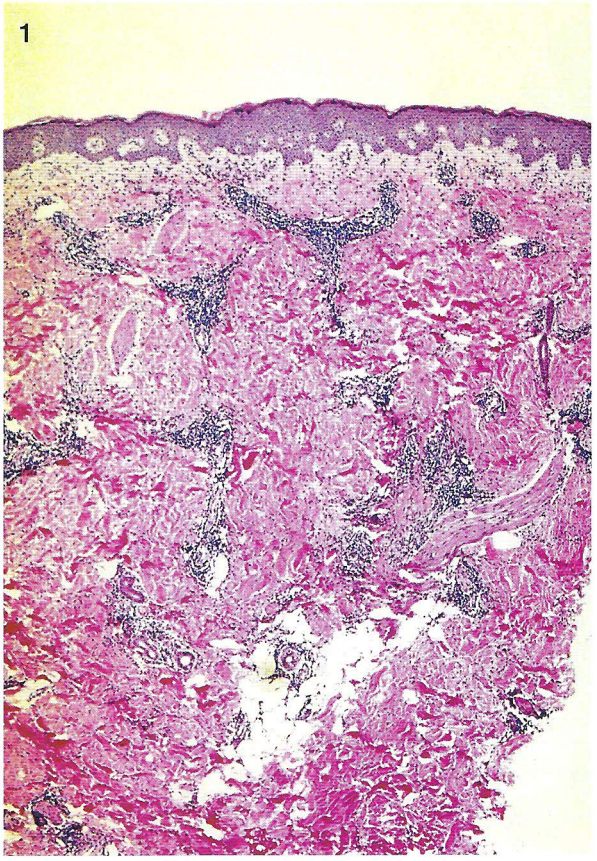


Figure 1. Erythema migrans. Moderate to severe superficial and deep mostly perivascular mononuclear infiltrate. Hematoxylin and eosin, x 10

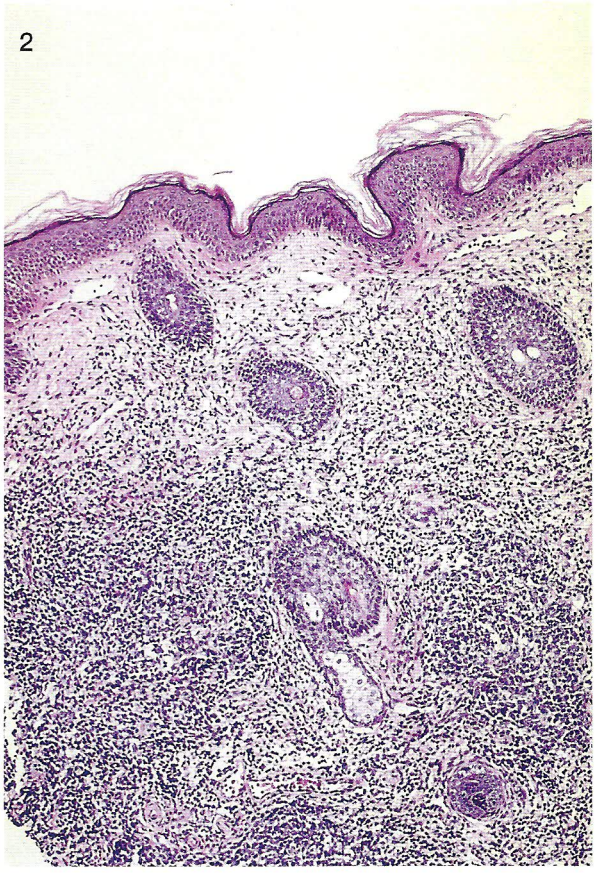


Figure 2. Follicular type of borrelial lymphocytoma with a dense infiltrate predominantly in the deep dermis. Hematoxylin and eosin, x 10

in the superficial part of the dermis and directly around the tick bite injury and is located around blood vessels as well as between collagen fibers. In addition, edema of the papillary dermis, extravasation of erythrocytes, and vascular dilatation can be observed in tick bite lesions (4-6).

Less important findings in EM, which are also not present in all cases, are a slight edema of the papillary (and reticular) dermis, loss of pilosebaceous units, an increased number of fibroblasts/fibrocytes, and fibrosis of the reticular dermis. The epidermis in EM lesions is usually intact, but a thickened basket-weave horny layer, atrophy and/or spongiotic foci may occur. Sometimes a lymphocytic infiltrate is located at the dermo-epidermal junction with disruption of the basement membrane (4).

Histopathology of borrelial lymphocytoma

BL is a lesion of cutaneous lymphoid hyperplasia (7). There are two histopathologic types of BL (5,6) with (follicular type) or without (diffuse/nodular type) follicular structures, resembling the germinal centers of lymph nodes (8). Combinations between the two types can be observed. (i) In the diffuse/nodular type, a dense infiltrate of mature lymphocytes, lymphoid cells with pale-staining indented or cleaved nuclei, sometimes in clusters, plasma cells, and sometimes eosinophils and multinucleated giant cells can be observed especially in the upper and mid dermis. There is a connective tissue background reaction with an increased number of fibroblasts, fibrosis, and edema. (ii) In the follicular type (Figure 2), there is a dense, nodular infiltrate in the deep dermis that may reach down into the panniculus. This infiltrate forms follicular structures with germinal centers, which consist of the same cells (polymorphic lymphoid cells) as in the germinal centers of normal lymphatic tissue, thus imitating secondary lymph node follicles. The infiltrate between the follicular structures is predominantly composed of small lymphocytes, plasma

Figure 3. Acrodermatitis chronica atrophicans. Clustered plasma cells within a perivascular infiltrate. Hematoxylin and eosin, x 40

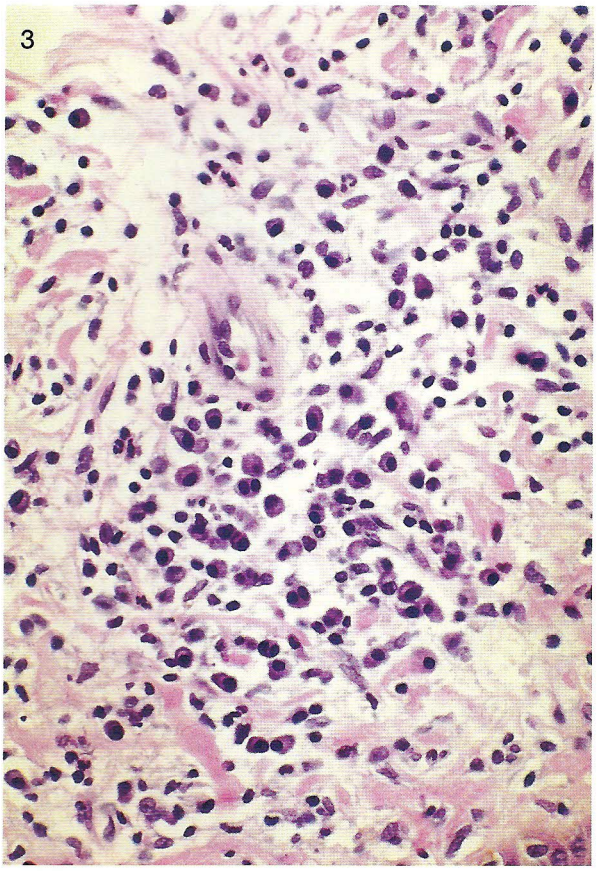
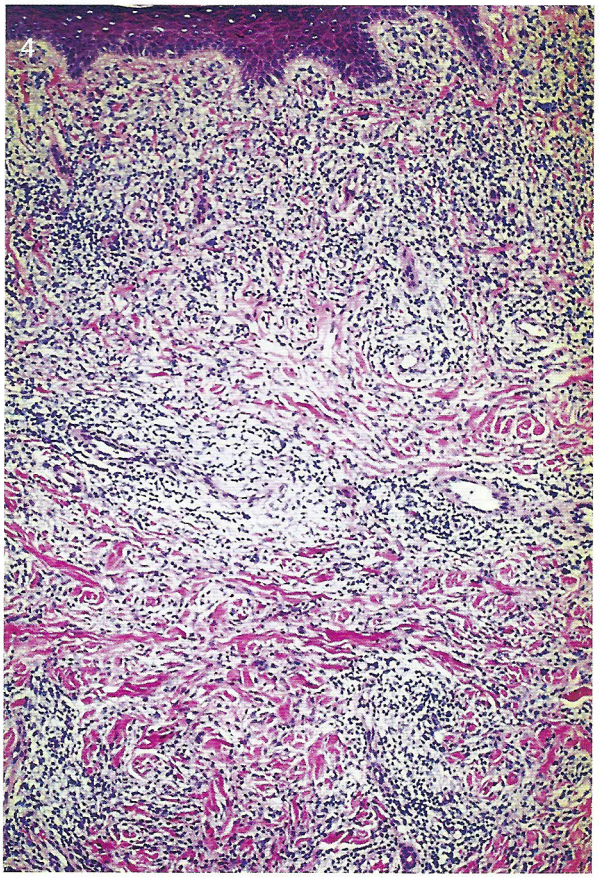


Figure 4. Acrodermatitis chronica atrophicans. Early lesion with dense patchy and subepidermal band-like infiltrate. Hematoxylin and eosin, x 10



cells, some eosinophils, mast cells, and histiocytes. Edema and dilated vessels are seen in the papillary dermis, where the infiltrate is lacking.

BL can be confused with well-differentiated nodular lymphoma histologically, but BL is always a pseudolymphoma, thus representing a benign lesion with a polyclonal proliferation of B and T cells (4,9). Important histopathologic differences to malignant lesions are a predominance of small lymphocytes, a mixed cell infiltrate, and lack of necroses. In difficult situations (e.g., predominance of lymphoid cells) it is necessary to prove polyclonality by immunohistochemistry (staining for Kappa and Lambda light chains) and PCR (T and B-cell rearrangement studies).

Histopathology of acrodermatitis chronica atrophicans

ACA develops clinically from an acute inflammatory phase into a chronic atrophic phase over many weeks to months without adequate antibiotic treatment. This clinical course is reflected by different histopathologic features with a predominance of infiltrative changes in the early stage and cutaneous atrophy in the late stage of the disease. A significant finding in ACA is a patchy to band-like mononuclear infiltrate that is pronounced in the superficial dermis but also present in the deep portion of the dermis and sometimes reaches down into the panniculus. The infiltrate is concentrated around blood vessels, which are often dilated, and skin appendages, but also extends between collagen fibers. It is composed of lymphocytes and histiocytes, and (clustered) plasma cells (Figure 3) in greater numbers than in EM. The severity of the infiltrate is usually moderate to severe in early lesions (Figure 4) and mild to moderate in older lesions (Figure 5), but even in the chronic phase the infiltrate is still notable. Only in very late burned-out lesions, the infiltrate is sparse or may even be lacking (13). Atrophy of the epidermis with thinning and loss of rete ridges develops over time (Figure 5). In the late stage, the epidermis is often only a few cell layers thick. Interface dermatitis with necrosis of basal

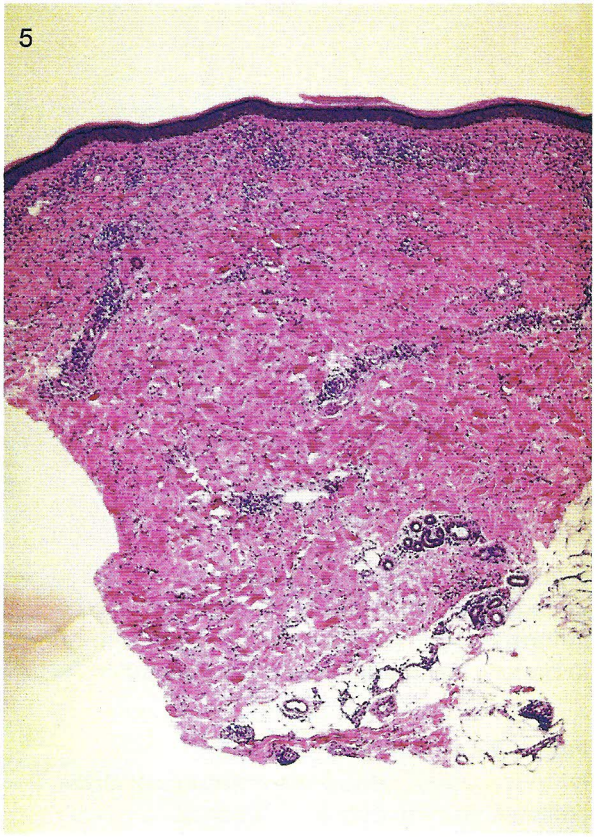


Figure 5. Acrodermatitis chronica atrophicans. Advanced lesion with mild superficial and deep dermal infiltrate and atrophy of the epidermis. Hematoxylin and eosin, x 10

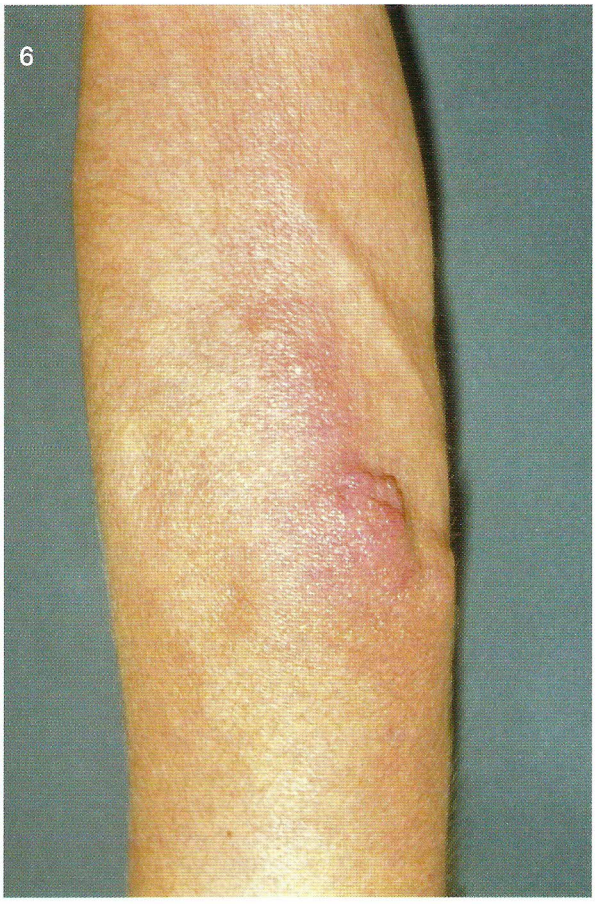


Figure 6. Fibrotic nodules over the elbow of a 45-year-old man.

cells may be seen in those cases where the dermal infiltrate is lichenoid band-like. An increased number of fibroblasts and fibrosis may be explained by the fact that Bb is commonly aligned with collagen fibrils and activates fibroblasts through cytokines. Fibrosis starts to develop in the early phase of the disease. Degeneration and marked reduction of collagen and elastic fibers, which accounts for a reduction of the breadth of the dermis, can be seen in the late stage. Elastic fibers are regularly surrounded by macrophages and multinucleated giant cells with elastophagocytosis, which may explain the loss of elastic fibers. This phenomenon can be observed on light microscopy (6) as well as on electron microscopy (11). Pilosebaceous units and sweat glands are first infiltrated and finally disappear as well. Another finding, which is less important, is an interstitial (mucinous) edema, which is often found in early lesions. Hauser (13) has described small fat vacuoles and fat cells in ACA lesions, but Brehmer-Andersson et al. (12), who described these structures as small groups or massive bands of vacuoles in the upper half of the dermis, have suggested that they represent rather lymphedema or lymphostasis.

Fibrotic nodules and sclerodermiform conditions in acrodermatitis chronica atrophicans

Fibrotic (syn.: fibroid, fibrous) nodules and/or pseudoscleroderma occur in 10% of ACA patients and are characterized by an increase in collagen in the dermis. Fibrotic nodules are dome-shaped nodules with a hard consistency in juxtaarticular locations, such as over the dorsal aspects of the elbows (Figure 6) or knees. Fibrotic nodules can be confused with rheumatoid nodules and gouty tophi clinically, and histopathology is necessary for the distinction of these conditions. The predominant finding is the presence of coarse, hyalinized collagen bundles (Figure 7) within the middle and deep part of the broadened dermis, extending into the

subcutaneous fat. The collagen fibers are arranged in peculiar onion-like concentric, or cartwheel-like, or interlacing patterns around a homogenous eosinophilic center. The number of fibroblasts is markedly increased within the entire dermis. A mild lympho-plasmacellular and histiocytic infiltrate, sometimes with eosinophils, is found in the upper dermis around blood vessels and accompanies the collagen formations in bands. The density of the infiltrate decreases towards the center of the collagen formations. The overlying epidermis is usually atrophic with some orthokeratosis. There is a complete loss of adnexal structures. Bb is well cultivable from fibrotic nodules (14).

Sclerodermiform (morphea-like) conditions, named pseudoscleroderma, can be found within ACA lesions, and appear clinically as whitish, ivory-colored indurated areas. Histopathologically, the epidermis is thinned and flattened in these areas. Homogenous hyalinized collagen is found within the excessively broadened dermis, and the number of fibroblasts, which entrap the collagen bundles, is markedly increased. Aggregates of lymphocytes and plasma cells are arranged around blood vessels but also interstitially. Eosinophils may be present within these infiltrates. The subcutaneous fat is largely replaced by collagen.

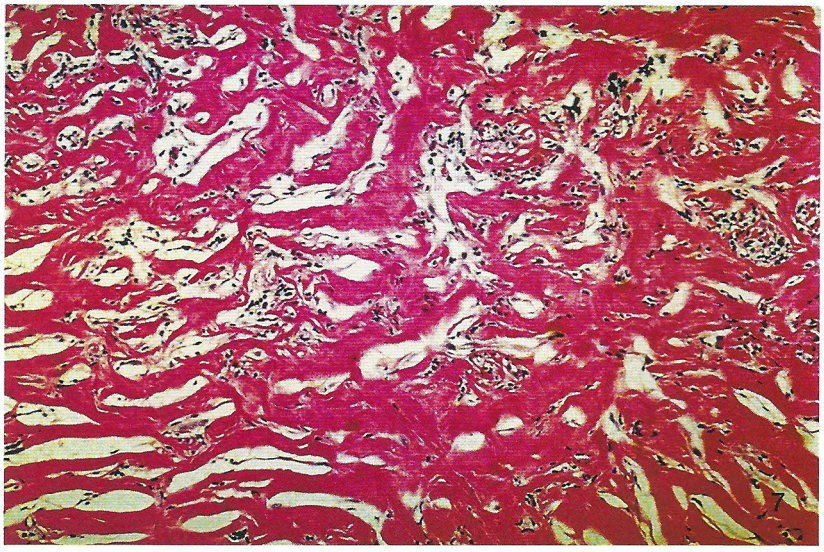
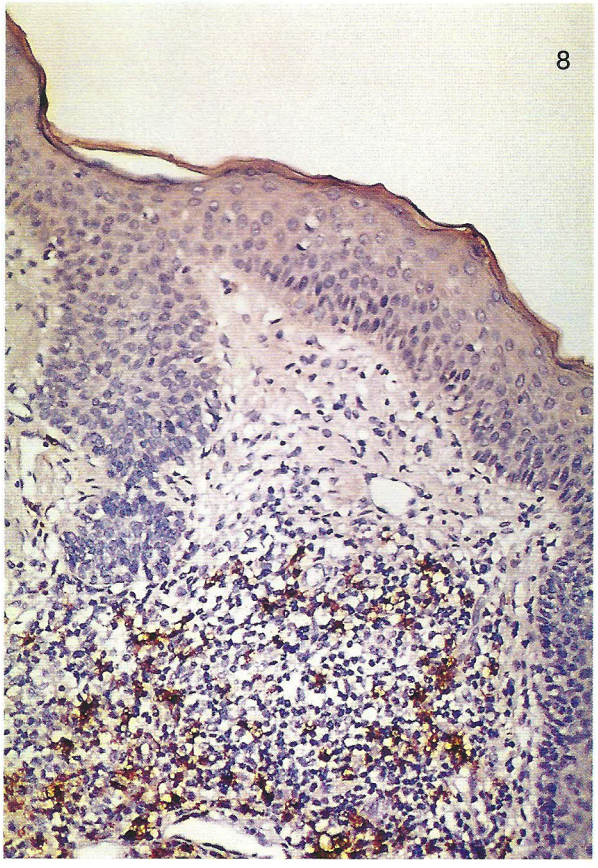


Figure 7. Fibrotic nodule. Coarse interlacing collagen bundles with mild mononuclear infiltrate accompanying the collagen formations in bands. Hematoxylin and eosin, x 10

Figure 8. Diffuse type of borrelial lymphocytoma. Moderate numbers of CD3+ T cells within perivascular infiltrates. Immunoperoxidase stained with anti-CD3 antibodies. x 25



Immunohistochemistry of dermatoborrelioses

We have analyzed biopsy specimens from 6 EM patients without extracutaneous signs and symptoms (EM minor), 6 EM patients with associated features (EM major), 5 BL patients, and 10 ACA patients for the expression of leukocyte differentiation antigens CD68 (macrophages), CD3 (T cells), CD4 (T-helper cells), CD8 (T-suppressor cells), and CD20 (B cells) using the avidin-biotin-peroxidase complex method (15). The percentage of cells positive for a respective leukocyte marker was estimated relative to the total number of infiltrating cells and positivity was graded on a scale from 0-3. The results were statistically compared between the patient groups (Table). Among the 6 patients with EM major, great numbers of macrophages were found; T cells were seen in moderate number, and B cells were infrequent. Among the 6 patients with EM minor, T cells were the predominant cell type, and they were present in significantly higher numbers than macrophages or B cells. When the two groups were compared, macrophages were detected significantly more often among EM major patients. In BL lesions, most of

the cells were B lymphocytes, but T cells (Figure 8) and macrophages were also present in significant numbers. In the 10 ACA patients, macrophages, T cells, and B cells were found in moderate numbers. CD8+ cells outnumbered CD4+ cells significantly in all patient groups. Two other examinations of leukocyte surface markers in DB have been performed so far. Büchner and Rufli (16) analyzed 9 patients with EM and found that the perivascular infiltrates within the erythematous peripheral portion of EM were composed predominantly of LEU4+ T cells (CD3). In contrast to our study, LEU3a+ helper cells (CD4) were more numerous than LEU2a+ suppressor T cells (CD8). The authors also found a high number of LEU6+ Langerhans cells in the epidermis and dermis in the same areas. Brehmer-Andersson et al. (12) have examined 7 patients with ACA by PAP immunoperoxidase staining and have found that the lymphocytes were T cells mainly expressing the UCHL1 antigen (CD45RO), whereas very few lymphocytes expressed B-cell antigen, despite the presence of numerous plasma cells. Corresponding to this considerable number of plasma cells in ACA, we found approximately the same number of B cells and T cells in our patients.

Conclusions

There are integrative and disintegrative histopathologic elements in DB. Common to all manifestations of DB is the presence of a lymphohistiocytic infiltrate intermingled with plasma cells (typical LB infiltrate) and a characteristic connective tissue reaction. Differences between the various manifestations of DB concern the pattern of the infiltrate, the proportion of the cell types within the infiltrate, the intensity of the infiltrate and the connective tissue reaction, and the presence or absence of epidermal changes. The integrative immunohistochemical elements in all forms of DB are the presence of a mixed mononuclear infiltrate, in which CD8+ cells always outnumber CD4+ cells. Differences concern the number of macrophages, which are found significantly more often in patients with EM major than in those with EM minor. Furthermore, T cells are prevalent in EM, whereas B cells are prevalent in BL.

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